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Kinetic Resolution of Racemic α -Aminonitriles via Stereoselective N-Acetylation Catalyzed by Lipase in Organic Solvent (Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occation of His Retirement)

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CITATION:

Nakai, Katsumi ...[et al]. Kinetic Resolution of Racemic α -Aminonitriles via Stereoselective N-Acetylation Catalyzed by Lipase in Organic Solvent (Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occation of His Retirement). Bul ...

ISSUE DATE:

1992-10-30

URL:

<http://hdl.handle.net/2433/77456>

RIGHT:

NOTE

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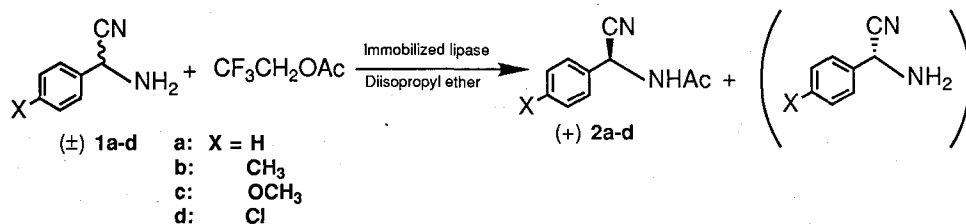
Received July 27, 1992

KEY WORDS: α -aminonitriles / Kinetic resolution / Stereoselective *N*-acetylation / Lipase-catalysis in organic solvent /

Lipase-catalysis in organic synthesis has received much attention as an effective method for the preparation of optically active alcohols, carboxylic acids and their derivatives¹. In particular, asymmetric esterification or transesterification in organic solvents is a method of frequent choice for facile kinetic resolutions of racemic alcohols². In the course of our studies on the kinetic resolution of racemic α -hydroxynitriles (cyanohydrins) via lipase-catalyzed acylation in an organic solvent³, we were interested in the kinetic resolution of their amino analogues, α -aminonitriles, via stereoselective *N*-acylation. α -Aminonitriles are important key intermediates for the synthesis of amino acids (Strecker synthesis)⁴ and are easily prepared from the corresponding cyanohydrins and ammonia⁵. We report here the stereoselective *N*-acetylation of racemic α -aminonitriles catalyzed by lipase in an organic solvent.

Racemic α -aminonitriles **1 a-d** were allowed to react with 2,2,2-trifluoroethyl acetate (TFEA) in anhydrous diisopropyl ether in the presence of an immobilized lipase (Scheme). The α -aminonitriles are unique in that they did not react with TFEA at all without the lipase⁶, probably due to the strong electron-withdrawing effect of the cyano group at the α -position. As a preliminary experiment, four different lipases⁷ including three *Pseudomonas* lipases from different origin and a lipase from *Chromobacterium viscosum* were tested for the *N*-acetylation of **1a**. As shown in Table 1, the enantioselectivity as well as the reaction rate was highly dependent on the lipase, although the total activity or the amount of the active lipase added did not differ significantly from each other⁸. Among the four lipases tested, a lipase from

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Table 1. Stereoselective *N*-acetylation of **1a** catalyzed by different lipases^a

| origin of lipase | supplier | amount of enzyme (unit) ^b | reaction time (h) | conversion ^c (%) | 2a | |
|---------------------------------|-----------|---|----------------------|--------------------------------|---------------------------|--------------|
| | | | | | e. e. ^d (%) | abs. config. |
| <i>Pseudomonas aeruginosa</i> | Toyobo | 10.5 | 44 | 53 | 72 | <i>S</i> |
| <i>Pseudomonas</i> sp. | Kurita | 17.0 | 147 | 42 | 32 | <i>S</i> |
| <i>Pseudomonas cepacia</i> | Amano | 28.4 | 147 | 53 | 10 | <i>R</i> |
| <i>Chromobacterium viscosum</i> | Toyo Jozo | 8.9 | 147 | 51 | 70 | <i>S</i> |

^aConditions: **1a** (50mg, 0.38mmol), 2,2,2-trifluoroethyl acetate (162mg, 1.14mmol), immobilized lipase powder (38mg), dry diisopropyl ether (10ml), 40°C.

^bThe catalytic activity of the lipases was measured by transesterification of 2,2,2-trifluoroethyl octanoate (TFEO, 50mM) and ethanol (200mM) in dry diisopropyl ether (10ml).⁸⁾ One unit of lipase is the amount of immobilized lipase which converts 1 μmol of TFEO to ethyl octanoate per min.

^cDetermined by ¹H NMR.

^dDetermined by ¹H NMR in the presence of Eu(hfc)₃.

Pseudomonas aeruginosa (Toyobo) exhibited the highest catalytic activity and stereoselectivity toward **1a**. Despite the total catalytic activity of twice as much as that of Toyobo enzyme, very slow reaction as well as low stereoselectivity was observed for a lipase from *Pseudomonas cepacia* (Amano), which is so far one of the most frequently used biocatalysts for organic synthesis⁹⁾. The preliminary experiment revealed that the stereoselectivity of the reaction was also affected by the choice of the acylating reagent and that TFEA served as a better acylating reagent with respect to the enantiomeric ratio (*E*=37)¹⁰⁾ than isopropenyl acetate (*E*=19) for the reaction with the Toyobo enzyme. Thus the lipase from *Ps. aeruginosa* (Toyobo) and TFEA were used for the reaction on a preparative scale.

The results of the stereoselective *N*-acetylation of **1a-d** were summarized in Table 2. The reaction was monitored with ¹H NMR and was stopped by filtering the enzyme powder when the proper conversion was attained. The filtrate was washed with dilute HCl to remove the unreacted amines **1a-d**, and the products **2a-d** were purified by column chromatography. The e. e. of **2a-d** was determined by ¹H NMR, using a chiral shift reagent, Eu(hfc)₃. The absolute configuration of **2a** was found to be *S* by comparing the sign of the optical rotation with that reported¹¹⁾. The absolute configuration of **2b-d** was tentatively assigned as *S* by correlating the configuration to the sign of optical rotation and to the relative intensity of two ¹H NMR peaks observed for *N*-acetyl proton in the presence of the chiral shift reagent. In the case of the corresponding oxygen analogues, (*S*)-cynohydrin acetates, the ¹H NMR

Lipase-catalyzed Kinetic Resolution of Racemic α -aminonitrilesTable 2. Kinetic resolution of (\pm)-**1a-d** via lipase-catalyzed *N*-acetylation^a

| α -amino nitriles 1a-d | reaction time (h) | conversion ^b (%) | Isolated yield of 2 (%) | $[\alpha]_D^{25}$ ^c (deg) | e. e. of 2 ^d (%) |
|---|----------------------|--------------------------------|-----------------------------------|---|---------------------------------------|
| 1a X=H | 19 | 40 | 40 | +16.0 | 90 |
| 1b CH ₃ | 56 | 43 | 42 | +24.5 | 86 |
| 1c CH ₃ O | 80 | 50 | 44 | +30.5 | 90 |
| 1d Cl | 147 | 39 | 24 | +32.6 | 87 |

^aTypical reaction conditions: α -aminoacetonitriles **1a** (6.0mmol), 2,2,2-trifluoroethyl acetate (18.0mmol), immobilized lipase powder (1.3g), dry diisopropyl ether (100ml), 40°C.

^bDetermined by ¹H NMR (200MHz).

^cc=1.0, CHCl₃.

^dDetermined by ¹H NMR in the presence of Eu(hfc)₃.

peak (OAc) in lower magnetic field was invariably larger³⁾, and this correlation holds for the *N*-acetyl proton of (*S*)-**2a** (see Experimental). The *S* configuration of **2a**, **c** and **2d** was also confirmed by converting them to the corresponding phenylglycines (6*N* HCl, reflux, 24 h) and by comparing the optical rotation of the amino acids with those reported^{12),13)}.

The lipase showed fairly good stereoselectivity for all the α -aminonitriles **1a-d**, giving optically active (*S*)-amides **2a-d** with 86 to 90% e. e. The stereochemical preference of the lipase for the α -aminonitriles was in good agreement with that observed for the corresponding oxygen analogue, mandelonitrile¹⁴⁾. Considering that efficient kinetic resolution of chiral amines has not yet been performed successfully by lipases¹⁵⁾, the present lipase served as an effective catalyst for the preparation of optically active amines.

EXPERIMENTAL

¹H and ¹³C NMR spectra were measured on a Varian VXR-200 spectrometer (200 MHz). Solvent was CDCl₃ with TMS as an internal standard. Infrared spectra were recorded on a Hitachi 215 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed on a Yanako MT-5 apparatus. Melting points are uncorrected. Diisopropyl ether was distilled over CaH₂ and stored over molecular sieves 4 Å. 2,2,2-Trifluoroethyl acetate (TFEA) was prepared from acetyl chloride and 2,2,2-trifluoroethanol [neat, 0°C to room temp., bp 79°C, 74%]. The substrates **1a-d** were prepared from the corresponding cyanohydrins by the reported procedure⁵⁾. The lipases are generous gifts from the companies as follows: *Pseudomonas aeruginosa* TE3285 (Toyobo Co., Ltd.; Osaka, Japan); *Pseudomonas cepacia* (Amano Pharmaceutical Co., Ltd.; Nagoya, Japan); *Pseudomonas* sp. KWI-56 (Kurita Water Industries Ltd.; Atsugi, Japan); *Chromobacterium viscosum* (Toyo jozo Co., Ltd.; Tokyo, Japan). The lipases were used after immobilization as described below.

Immobilization of lipase.

The lipase (10 mg) and sucrose (75 mg) were dissolved in 20mM Tris-HCl buffer (pH 8.0, 5 ml). Hyflo Super-Cel (Johns-Manville International Corp., 1 g) was added to the solution, and the mixture was stirred at 0°C for 15min and lyophilized. The lyophilized powder was slightly moistened by exposing the powder to humid air (37 °C, 24 h). This operation was essential for high catalytic activity of the immobilized lipase in an organic solvent.

Preparation of (S)-(+)-N-(1-cyano-1-phenyl)methyl acetamide (2a);

Typical procedure. 2-Amino-2-phenylacetonitrile (**1a**) (1.0 g, 7.6 mmol) and 2,2,2-trifluoroethyl acetate (3.2 g, 23 mmol) were dissolved in dry diisopropyl ether (100 ml). The immobilized lipase (1.3 g) was added to the solution. The suspension was stirred for 19h at 40 °C. The reaction conversion was checked by ¹H NMR [CH proton for **1a** (δ =4.92, s), and CH and NH proton for the amide **2a** (δ =6.09–6.23, m)] and was found to be 40%. The reaction mixture was filtrated and the lipase powder was washed three times with diisopropyl ether. The combined filtrates were washed with 2*N* HCl and brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was chromatographed on silica gel [dichloromethane (19): ethyl acetate (1)] to afford (S)-(+)-N-(1-cyano-1-phenyl)methyl acetamide (**2a**) as colorless crystals (531 mg, 40% yield); mp 100.3 °C; $[\alpha]_D^{25} = +16.0^\circ$ (*c* 1.00, CHCl₃) [lit.¹¹]. $[\alpha]_D^{25} = -21.65^\circ$ (*c* 1.4, CHCl₃) for the *R* isomer with 100 % e. e.]; 90 % e. e. [¹H NMR with Eu(hfc)₃: δ (NHCOCH₃) 5.48 (*R*, minor) and 5.80 (*S*, major)]; ¹H NMR δ =2.07 (s, 3H, CH₃CO), 6.09–6.23 (m, 2H, CH and NH), 7.41–7.52 (m, 5H_{arom}); IR (KBr) 2200 (C≡N) and 1640 (C=O) cm⁻¹; ¹³C NMR δ =22.57 (CH₃CO), 44.11 (CH), 117.55 (C≡N), 127.04, 129.33, 129.51, 133.12, and 168.9 (C=O); Anal. Calcd. for C₁₀H₁₀N₂O: C, 68.95; H, 5.78; N, 16.08 %. Found: C, 69.04; H, 5.85; N, 15.76 %.

(S)-(+)-N-[1-cyano-1-(4-methylphenyl)]methyl acetamide (2b).

Prepared from 2-amino-2-(4-methylphenyl)acetonitrile (**1b**) in 43 % conversion yield. Column chromatography [dichloromethane (19); ethyl acetate (1)] gave **2b** as colorless crystals: (42 %); mp 158.7–159.2 °C; $[\alpha]_D^{25} = +24.5^\circ$ (*c* 1.00, CHCl₃); 86 % e. e. [¹H NMR with Eu(hfc)₃: δ (NHCOCH₃) 5.78 (minor) and 6.14 (major)], the absolute configuration was assigned as *S* by correlating the configuration to the sign of optical rotation and to the relative intensity of the two ¹H NMR peaks shown above; ¹H NMR δ =2.06 (s, 3H, CH₃CO), 2.38 (s, 3H, CH₃), 5.96–6.10 (m, 2H, CH and NH), 7.22–7.39 (m, 4H_{arom}); IR (KBr) 2200 (C≡N) and 1640 (C=O) cm⁻¹; ¹³C NMR δ =21.14 (CH₃), 22.69 (CH₃CO), 43.90 (CH), 117.63 (C≡N), 126.96, 129.99, 130.20, 139.65, and 169.41 (C=O); Anal. Calcd. for C₁₁H₁₂N₂O: C, 70.19; H, 6.42; N, 14.88%. Found: C, 70.40; H, 6.40; N, 14.90%.

(S)-(+)-N-[1-cyano-1-(4-methoxyphenyl)]methyl acetamide (2c).

Prepared from 2-amino-2-(4-methoxyphenyl)acetonitrile (**1c**) in 50 % conversion yield. Column chromatography [dichloromethane (19): ethyl acetate (1)]

gave **2c** as colorless crystals: (44%); mp 161.1–161.2 °C; $[\alpha]_D^{25} = +30.5^\circ$ (c 1.00, CHCl₃); 90% e.e. [¹H NMR with Eu(hfc)₃; δ (NHCOCH₃) 5.14 (minor) and 5.46 (major)], the absolute configuration was assigned to be S by the same correlation as for (S)-(+)–**2b**; ¹H NMR δ =2.05 (s, 3H, CH₃CO), 3.83 (s, 3H, CH₃O), 6.01–6.96 (m, 2H, CH and NH), 6.92–7.42 (2×d, J =8.8 Hz, 4H_{arom}); IR (KBr) 2200 (C≡N) and 1640 (C=O); ¹³C NMR δ =22.74 (CH₃CO), 43.66 (CH), 55.42 (CH₃O), 114.69, 117.67 (C≡N), 125.14, 128.48, 160.44, and 169.24 (C=O); Anal. Calcd. for C₁₁H₁₂N₂O₂: C, 64.69; H, 5.92; N, 13.72%. Found: C, 64.81; H, 5.86; N, 13.67%.

(S)-(+)–N-[1-cyano-1-(4-chlorophenyl)]methyl acetamide (2d).

Prepared from 2-amino-2-(4-chlorophenyl)acetonitrile (**1d**) in 39% conversion yield. Column chromatography [dichloromethane (19): ethyl acetate (1)] gave **2d** as colorless crystals: (24%); mp 129.1–129.2 °C; $[\alpha]_D^{25} = +32.6^\circ$ (c 1.00, CHCl₃); 87% e.e. [¹H NMR with Eu(hfc)₃; δ (NHCOCH₃) 5.68 (minor) and 6.20 (major)]; the absolute configuration was assigned to be S by the same correlation as for (S)-(+)–**2b**; ¹H NMR δ =2.07 (s, 3H, CH₃CO), 6.28 (m, 2H, CH and NH), 7.41 (m, 4H_{arom}); IR (KBr) 2200 (C≡N) and 1640 (C=O); ¹³C NMR δ =22.68 (CH₃CO), 43.48 (CH), 117.16 (C≡N), 128.41, 129.54, 131.69, 135.66, and 169.65 (C=O). Anal. Calcd. for C₁₀H₉ClN₂O: C, 57.57; H, 4.35; N, 13.43%. Found: C, 57.73; H, 4.38; N, 13.12%.

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- 6) In the absence of lipase, no appreciable *N*-acetylation was detected at 40°C after 3 days.
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- 8) The specific activity of each immobilized lipase was measured by the transesterification of 2,2,2-trifluoroethyl octanoate and ethanol in anhydrous diisopropyl ether [T. Nakatani, J. Hiratake, K. Yoshikawa, T. Nishioka, and J. Oda, *Biosci. Biotech. Biochem.*, **56**, 1118 (1992)].
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